

We claim:

1. A method for detecting the proximity of a first molecular segment to a second molecular segment, comprising:
  - a) covalently attaching fluorescein to a first molecular segment;
  - b) covalently attaching cyanine 5 to a second molecular segment; and
  - c) detecting the presence or absence of fluorescein-induced emission of cyanine 5 as a result of fluorescence resonance energy transfer when the first molecular segment and second molecular segment are in proximity to each other.
2. The method of claim 1, wherein the method is used as part of a fluorescence resonance energy transfer-based assay.
3. The method of claim 2, wherein the resonance energy transfer-based assay is a heterogeneous assay.
4. The method of claim 2, wherein the resonance energy transfer assay is a homogenous assay.
5. The method of claim 1, wherein the first molecular segment and second molecular segment are parts of the same molecule.
6. The method of claim 1, wherein the first molecular segment and second molecular segment are not parts of the same molecule.
7. The method of claim 6, wherein the first molecular segment is a part of a first member of a binding pair or a part of a first linker moiety that is directly or indirectly attached to the first member of the binding pair, and the second molecular segment is a

part of a second member of a binding pair or a part of a second linker moiety that is directly or indirectly attached to the second member of the binding pair.

5           8. The method of claim 7, wherein the first molecular segment is a part of the first member of the binding pair, and the second molecular segment is a part of the second member of the binding pair.

10           9. The method of claim 7, wherein the first molecular segment is a part of a first linker moiety that is directly or indirectly attached to the first member of the binding pair, and the second molecular segment is a part of a second linker moiety that is directly or indirectly attached to the second member of the binding pair.

15           10. The method of claim 9, wherein the first or second linker moiety is independently selected from the group consisting of an antibody, antibody fragment, biotin and streptavidin.

20           11. The method of claim 7, wherein the binding pair comprises an enzyme-enzyme substrate pair.

25           12. The method of claim 7, wherein the binding pair comprises an antibody-antigen pair.

          13. The method of claim 7, wherein the binding pair comprises a biological receptor-ligand pair.

          14. The method of claim 7, wherein the binding pair comprises complementary oligonucleotides.

15. The method of claim 1, wherein the proximity is about 1 Å to about 100 Å.

16. The method of claim 15, wherein the proximity is about 5 Å to about 80 Å.

5 17. The method of claim 16, wherein the proximity is about 10 Å to about 70 Å.

18. The method of claim 17, wherein the proximity is about 20 Å to about 60 Å.

10 19. The method of claim 1, wherein the fluorescein-induced emission of cyanine 5 is detected using a 682 peak emission filter.

20. The method of claim 1, wherein the total number of first and second molecular segments is from 2 to about 12.

15 21. The method of claim 20, wherein the total number of first and second molecular segments is from 2 to about 8.

22. The method of claim 2, wherein the fluorescence resonance energy transfer-based assay is used to determine affinity of ligand to a receptor.

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23. The method of claim 2, wherein the fluorescence resonance energy transfer-based assay is used to detect a target molecule selected from the group consisting of a protein, an RNA, a DNA, an antigen and an antibody.

25 24. The method of claim 2, wherein the resonance energy transfer-based assay is used to determine the structural conformation of a molecule.

25. The method of claim 2, wherein the resonance energy transfer-based assay is used to monitor enzymatic reactions.

*Sub A1*  
26. A composition comprising a first member of a binding pair directly or indirectly attached to fluorescein and a second member of the binding pair directly or indirectly attached to cyanine 5, wherein the first and second members of the binding pair are associated so that the fluorescein and cyanine 5 are in fluorescence resonance energy transfer proximity to each other.

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27. The composition of claim 26, wherein the first member of the binding pair is directly attached to fluorescein and the second member of the binding pair is directly attached to cyanine 5, and the direct attachment is effected through a covalent bond.

15 28. The composition of claim 26, wherein the first member of the binding pair is indirectly attached to fluorescein and the second member of the binding pair is indirectly attached to cyanine 5, and the indirect attachment is effected through one or more linking moieties.

20 29. The composition of claim 26, wherein at least one member of the binding pair is indirectly attached to either fluorescein or cyanine 5, and the indirect attachment is effected through one or more linking moieties.

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25 ~~30. The composition of claim 26, wherein the binding pair comprises an enzyme-enzyme substrate pair.~~

31. The composition of claim 26, wherein the binding pair comprises an antibody-antigen pair.

32. The composition of claim 26, wherein the binding pair comprises a biological receptor-ligand pair.

33. The composition of claim 26, wherein the binding pair comprises complementary oligonucleotides.

34. The composition of claim 26, wherein the fluorescence resonance energy transfer proximity is about 1 Å to about 100 Å.

35. The composition of claim 34, wherein the fluorescence resonance energy transfer proximity is between about 5 Å to about 80 Å.

36. The composition of claim 35, wherein the fluorescence resonance energy transfer proximity is between about 10 Å to about 70 Å.

37. The composition of claim 36, wherein the fluorescence resonance energy transfer proximity is between about 20 Å to about 60 Å.

38. A compound comprising a first molecular segment covalently bound to fluorescein and a second molecular segment covalently bound to cyanine 5.

39. The compound of claim 38, wherein the fluorescein and cyanine 5 are in fluorescence resonance energy transfer proximity to each other.

40. The compound of claim 39, wherein the fluorescence resonance energy transfer proximity is about 1 Å to about 100 Å.

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41. The compound of claim 40, wherein the fluorescence resonance energy transfer proximity is between about 5 Å to about 80 Å.

42. The compound of claim 41, wherein the fluorescence resonance energy transfer proximity is between about 10 Å to about 70 Å.

43. The compound of claim 42, wherein the fluorescence resonance energy transfer proximity is between about 20 Å to about 60 Å.

44. In a FRET-based assay having a dye pair, the improvement comprising using fluorescein and cyanine 5 as the dye pair.

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